3210-Pos Board B315
The SR Calcium Content of Fast Muscle Fibres Lacking Calsequestrin is Reduced and not Sufficient for Sustained Contractions
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Calsequestrin (CASQ) is an acidic, high capacity Ca^{2+}-binding protein located within the terminal cisternae of the sarcoplasmic reticulum (SR). CASQ1 is the major SR Ca^{2+}- buffer in fast muscle fibers, whereas CASQ2 predominates in cardiomyocytes and slow muscle fibers. Mice devoid of CASQ were generated by crossing mice lacking CASQ1 (Paolini et al J Physiol 2007, 583, 767) and mice lacking CASQ2 (Knollman et al J Clin Inv 2006, 116, 2510).
When studied ex vivo, fast muscles (EDL) showed an earlier decline in tension during tetanic stimulation than WT fibers. This was not the case in slow muscle, soleus. Such decline of the developed tension was compatible with the decay of free cytosolic calcium during repeated stimulation observed using Fura-2 in single intact fast FDB muscle fibers.
Single permeabilized fibers from EDL and tibialis anterior showed a reduction of the amount of calcium released by 30 mM caffeine, which is supposed to release about 80% of SR calcium (Murphy et al. J Physiol 2009, 587, 443), thus suggesting a depletion of SR. The depletion was confirmed by measurements of SR fee $[Ca^{2+}]$ using a FRET-based indicator (D1ER) genetically targeted to the SR. During contraction a massive/marked reduction in intraluminal free $[Ca^{2+}]$ was observed to a level close to full depletion.The results are consistent with the function of CASQ as a intraluminal SR buffer. CASQ1/2 null mice lack sufficient compensation for the loss in buffering power by other SR proteins. The diversity between slow and fast muscles might be attributed to the presence of parvalbumin as a relevant sink of calcium in cytosol of fast but not slow muscles (Celio et al Nature 1982, 297, 504).

3211-Pos Board B316
Activity-Dependent Regulation of Mitochondrial Superoxide Flashes in Skeletal Muscle
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Reactive oxygen species (ROS) are a class of molecules that oxidize diverse cellular proteins/lipids and are generated mainly in mitochondria by the electron transport chain (ETC). We previously discovered a novel biosensor for superoxide, circularly permutated YFP (cpYFP). Using mitochondrial-targeted cpYFP (mt-cpYFP), localized bursts of superoxide in individual or clusters of mitochondria, termed "superoxide flashes," are observed in quiescent cells across a wide range of cell types [Wang, W. et al., *Cell* 134:279-290, 2008]. Here, we examined the properties of superoxide flashes in *flexor digitorum brevis* muscle fibers from newly generated transgenic mice expressing skeletal muscle-specific mt-cpYFP. A new flash detection and analysis software ("Flash Collector") was developed to enable automated quantification of flash frequency, amplitude, kinetics, and area. Results demonstrate that skeletal muscle fibers exhibit higher basal mitochondrial superoxide flash frequency, but similar amplitude, kinetics, and area as those observed in cardiac myocytes. Rotenone (5υM) and oligomycin (5υM) reduced flash frequency to ~20% of control, confirming the ETC dependence of superoxide flash generation. Inhibition of adenine nucleotide translocase by bongkrekic acid (100υM) decreased flash frequency by 50%. Incubation in Ringer's containing 10mM glucose or mitochondrial substrates did not significantly alter flash frequency, but increased flash amplitude and duration by 10-15%. Importantly, superoxide flash activity was enhanced (frequency increased from 18.1 ± 1.6 to 22.3 ± 2.0 flashes/100s·100υm^2) following five consecutive brief tetani (500ms, 100Hz, 0.2 duty cycle), but was markedly suppressed following a prolonged fatiguing stimulation (40 tetani; frequency reduced from 17.6 ± 2.2 to 7.7 ± 1.6). These results demonstrate skeletal muscle activity regulates mitochondrial superoxide flash production. In adition, muscle-specific mt-cpYFP transgenic mice will be a powerful tool for assessing the physiological role of superoxide flash activity and how this activity is altered and contributes to skeletal muscle aging and disease.

Actin & Actin-binding Proteins II

3212-Pos Board B317
Allosteric Effects within the Catalytic Domain of Dictyostelium Myosin on Interaction with Actin and Nucleotide
Piyali Guhathakurta, Ewa Prochniewicz, Joseph M. Muretta, David D. Thomas.
To understand molecular features of myosin critical for its functional interaction with actin, we studied two constructs of Dictyostelium myosin II, which differ in cysteine content and length: (i) Cys-lite catalytic domain (M758-S619C) and (ii) fusion of wild type catalytic domain with two α-actinin repeats (M761-2R), which has been shown to be kinetically identical to full-length Dictyostelium myosin S1. In the absence of ATP, both constructs bound actin stoichiometrically. However, marked differences in interaction of each construct with actin were observed in the presence of ATP. Actin-activated ATPase of M758-S619C was very sensitive to the presence of an ATP regenerating system (i.e., elimination of free ADP), which increased activity by a factor of 2.5, while M761-2R was not affected. The ATP regenerating system greatly decreased actin binding of M758-S619C, as measured by cosedimentation. Fluorescence resonance energy transfer between IAEDANS-actin and IAF-M758-S619C showed that this interaction was strong in the presence of ATP, independent of ionic strength, and structurally indistinguishable from that of the strong-binding complex. These results indicated that M758-S619C has an altered relationship between the binding of actin and nucleotide. Binding of MantADP confirmed this hypothesis: actin had no significant effect on the tight binding ($K_d \sim 1$ μM) of MantADP to M758-S619C, while actin substantially decreased the affinity of MantADP for M761-2R, as for muscle S1. We conclude that the structural alterations of the M758-S619C construct (Cys content and/or the structure of the converter domain) allosterically modulates the reciprocal affinity of myosin for nucleotide and actin, which in turn changes the distribution of actomyosin states within the actomyosin ATPase cycle.

3213-Pos Board B318
Expression and Characterization of Full Length Nonmuscle Myosin IIs
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The nonmuscle myosin IIs (NMIIs) are filamentous myosins which are involved in a wide variety of cellular processes from cytokinesis to control of cell morphology. There are 3 isoforms of the NMII heavy chain in humans, NMIIA, IIB and IIC which are expressed at varying levels according to cell type. Owing to the difficulty of expressing the full length proteins, previous studies on this myosin have used tissue purified protein or expressed fragments of the molecule and in both cases this presents potential drawbacks for characterizing the protein *in vitro*. To circumvent current limitations and approach their native properties, we have successfully expressed full length wild type and mutated NMII proteins using the Sf9-baculovirus system. We also expressed two chimeric NMII proteins (one with the amino-terminal globular head of the IIA heavy chain fused to the rod of IIB; another with the IIB globular head preceding the IIA rod). We have begun characterizing the 3 human isoforms, along with their alternatively spliced counterparts and also mutants which are known to be associated with disease states in humans. Here we show that although full length NMIIA, IIB and IIC exhibit differences biochemically, the structure of the filaments determined by negative stain electron microscopy is essentially indistinguishable between the three isoforms. The ability of all 3 isoforms to adopt the 10S compact conformation in the presence of ATP is demonstrated. We also show using the chimeric constructs that the tail domains of different isoforms are interchangeable in terms of filament formation and formation of the 10S compact conformation.

3214-Pos Board B319
Mouse Models of Human *MYH9*-Related Diseases
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Point mutations in *MYH9*, the gene encoding nonmuscle myosin heavy chain IIA (NMHC IIA), underlie autosomal dominant syndromes in humans (incidence 1 in 500,000). The abnormalities can manifest as macrothrombocytopenia, granulocyte inclusions, progressive proteinuric renal disease, cataracts, and sensorineural deafness. To gain insight into the pathological mechanism of *MYH9*-related diseases in humans, we generated mouse models of three disease-associated mutations, Arg702Cys in the amino-terminal domain of NMHC IIA which controls myosin motor activity, and Asp1424Gln and Glu1841Lys in the carboxyl-terminal rod domain, which regulates filament formation. Heterozygous Asp1424Gln and Glu1841Lys mutant mice produce homozygous mutant offspring at close to normal ratios. By contrast, homozygous Arg702Cys mice die at embryonic day E10.5 to E11.5 which though early, is considerably later in development than knockout NMHC IIA mice (E6.5). These results indicate that the motor domain function of NMHC IIA is critically important during the latter phase of mouse embryonic development. Giant platelets accompanied by decreased platelet counts and prolonged bleeding times are found in adult heterozygous and homozygous mice from all three mutant mouse lines. Bone marrow histology is consistent with failure of platelet release into the circulation. Some adult heterozygotes from all three mouse lines and homozygotes from Asp1424Gln and Glu1841Lys mouse lines have higher urine albumin/creatinine ratios than those of wild type